Abstracts for the International Workshop on Neurobiology of the Skin

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NEUROGENIC INFLAMMATION: MECHANISMS AND PATHOPHYSIOLOGICAL IMPLICATIONS.

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The ability of a subset of primary sensory neurons (with C and Aδ fibres) to release neuropeptides from their peripheral endings, thereby causing local inflammatory responses has been referred to as 'neurogenic inflammation'. These neurons are uniquely sensitive to capsaicin and contains the neuropeptides substance P (SP), neurokinin A (NKA) and calcitonin gene-related peptide (CGRP). Neurogenic inflammatory responses caused by SP and NKA in a variety of tissues are arteriolar dilatation (hyperemia) and leakage of plasma protein and leukocyte adhesion in the postcapillary venules; CGRP mainly mediate arteriolar vasodilatation. SP, NKA and CGRP may also cause tissue-specific effects, (e.g., bronchoconstriction or positive inotropic and chronotropic effects). Release of neuropeptides from primary sensory neurons may be caused by axon reflexes or by a local effector mechanism. Neurogenic inflammatory mechanisms have been proposed to have a role in a variety of human diseases, including migraine, asthma and others. The flare response observed in the human skin, being inhibited by capsaicin pretreatment and by local anesthetics is considered to be due to neuropeptide released *via* an axon reflex mechanism. Capsaicin desensitization has been used successfully in some skin diseases. The recent development of high affinity and orally active non peptide antagonists for receptors of SP and NKA (NK_1 and NK_2 receptors) will allow to test the possible pathophysiological role of neurogenic inflammation in human pathology.

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SKIN INNERVATION AND NEUROGENIC INFLAMMATION

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Traditionally, cutaneous innervation has always been divided into an afferent and an efferent component. Sensory function transmits impulses from a wide array of peripheral specialized terminals to the central nervous system, providing information about the external environment and the skin own internal conditions. The efferent function, exerted by the sympathetic innervation, mainly contributes to our thermal homeostasis through its vascular and glandular connections. This time-honoured distinction, based on anatomical and functional knowledge, should be reconsidered on the basis of several experimental evidences that indicate an efferent role for sensory nerve fibers during cutaneous inflammatory responses. Indeed, several subsets of cutaneous nerve fibers are now recognized, differentiated according to their cutaneous distribution and relationships, as well as for their neurotransmitter content. Recent anatomical, biochemical and pharmacological data suggest specific regulatory functions exerted by different subsets of fibers. A neurogenic component must therefore be considered among the basic modulatory mechanisms in both physiologic and pathologic conditions.

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NEURO-EPITHELIAL INTERACTIONS IN HAIR GROWTH CONTROL.

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Clinical and experimental observations have long suggested that skin nerves have „trophic" functions in hair follicle (HF) development, growth and/or cycling. Using the murine hair cycle as an excellent system for characterizing and manipulating piloneural interactions, it is reported that both the sensory and the autonomic innervation of normal pelage HFs, the substance P and beta-endorphin content, tachykinin-degrading enzyme activity, mast cell-nerve contacts, and NCAM expression on cutaneous nerves all display striking changes during synchronized HF cycling in C57BL/6 mice. Furthermore, the HF is both a significant source and target of neuritrophins like NGF, for which defined follicle regions show hair cycle-dependent expression of high and low affinity receptors (TrkA, p75NTR). During the induced hair cycle, levels of NGF protein rise substantially in early anagen, which is associated with hyperinnervation of HFs, and then decline towards catagen. In murine skin organ culture, NGF modulates HF keratinocyte proliferation and retards catagen development. Selected neuropharmacological manipulations alter murine HF cycling in vivo. For example, substance P s.c. implants or ACTH, but not CGRP, induce anagen, as does the release of endogenous neuropeptides from sensory nerves by capsaicin, and of neurotransmitters from adrenergic nerves by 6-OH-dopamine or guanethidine. Both substance P and capsaicin i.c. also induce premature, but dystrophic catagen development in anagen skin in vivo. Taken together, this sugggests that neuroepithelial interactions are regulatory elements in hair growth control, and targeted manipulations of these interactions may become a novel strategy for managing hair growth disorders.

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THE MERKEL CELL: STATE OF THE ART

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Merkel cells (MC) are the neuroendocrine cells of the skin present in the basal epithelial layer. Ultrastructurally they are characterized by dense-cored granules and loosely arranged citoskeletal filaments, which are made up by cytokeratin (CK) polypeptides nos. 8, 18, 19 and 20. CK 20, helped to demonstrate that they are present in embryonic skin of week 8 exclusively within the epidermis. This suggested intraepidermal development of MC to which further support was lent by a mouse-human xenograft model. MC were detectable in the upper dermis only after week 14 showing a high concentration around developing sweat glands and hair follicles. Thus they may be involved in fetal adnexal growth. In hair follicles of adults MC are numerous within the bulge and isthmus where stem cells are located. Moreover their number is elevated around various epithelial tumors. This supporting the hypothesis that they may be involved in growth. CgA may be an important factor in this aspect, as it is expressed in 75% MC. Other relevant factors may be NGF, SP, VIP or others which are stored within dendrites. Further functions are the slowly adapting mechanoreception of the MC present within Pinkus-Haarscheiben of various mammals and men. Indeed, in murine skin the MC are restricted to these tiny organs, and their numbers vary for still unknown reasons dependent on the hair cycle with a maximum in anagen. In contrast to rodents human trunks skin contains, outside Haarscheiben, further 23 MC/mm^2 with a high elevation in chronically UV-damaged skin. This argues, for a certain turn over of MC in human skin; it is possible that they undergo apoptosis, what is in line with the bcl2-positivity of about 50% of MC in humans. Their renewal may most probably start from basal keratinocytes, similar to fetal skin, because MC do not express the proliferation marker Ki-67. Thus, despite knowledge of various details, important biological features of MC still wait for clarification.

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NEUROPEPTIDES AND THE IMMUNE SYSTEM. J.C. Ansel[1], KL Quinlan[1], I-S. Song[1], Bunnett NW[2], Olerud JE[3], Armstrong CA[1], Caughman SW[1], [1]Depart of Dermatology, Emory Univ School of Medicine, Atlanta, GA; [2]Departs of Physiology and Surgery, UCSF, San Francisco, CA; [3]Depart of Dermatology, Univ of Washington, Seattle, WA.

There is increasing evidence that skin inflammation can be mediated by the cutaneous neurologic system through the release of neuropeptides such as substance P (SP) that interact with target cells in the skin. SP has been well characterized as a potent vasodilator that can cause increased microvascular permeability and protein extravasation. Additionally, SP can induce leukocyte effector activities such as lymphocyte proliferation, cytotoxicity, and immunoglobulin production; mast cell degranulation; macrophage and polymorphonuclear leukocyte activation; and induction of cytokine production by monocytes. Our previous studies indicate that SP can directly activate mast cells and keratinocytes to secrete TNFα and IL-1, respectively. We have recently examined the effect of SP on cutaneous microvascular endothelial cell adhesion molecule and cytokine expression. Our results indicate that human dermal microvascular endothelial cells (HDMEC) express mRNA for three neurokinin receptors, NK-1R, NK-2R, and NK-3R, which are capable of binding to SP with high, intermediate, and low affinity, respectively. We demonstrate that SP induces a rapid intracellular Ca^{2+} response in HDMEC. This activation is further accompanied by increased levels of IL-8 and ICAM-1 mRNA and IL-8 secretion and ICAM-1 cell surface expression. In parallel, increased leukocyte binding is observed in SP-treated HDMEC. A similar induction of dermal microvascular endothelial cell ICAM-1 expression is observed *in vivo* after the topical application of the SP-releasing agent capsaicin. These studies significantly expand our understanding of the role of the cutaneous neurological system in inflammatory responses in the skin and will provide the foundation upon which novel anti-inflammatory therapeutic agents can be developed.

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Neuropeptides in contact dermatitis.

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Neuropeptides in sensory nerves, like substance P, neurokinin A and calcitonin gene-related peptide, participate in the protection of the body from noxious agents (nociception). C-fibers affect vasculature locally through axon reflexs, resulting in neurogenic inflammation. Neuropeptides stimulate mast cell degranulation and attract immuno-competent cells like Langerhans cells and lymphocytes. Peptidergic nerve fibers around lymph nodes create also a structural basis for communication with the immune system. Upon contact with allergic and irritant agents, several transmitters are released from cutaneous nerve fibers. Repeated administration of capsaicin, the main agent in hot pepper, depletes neuropeptides from Cfibers. Pretreatment with capsaicin has been shown to suppress the immediate allergic reaction and augment both the sensitisation and elicitation phase of delayed allergic reaction. The role of individual neuropeptides has been studied in various ways. Substance P seems to stimulate allergic immediate and delayed hypersensitivity reactions while having no effect on non-immunologic reactions. Calcitonin gene related peptide has been shown to inhibit the antigen-presenting capacity of Langerhans cells and to inhibit delayed hypersensitivity reactions. Vasoactive intestinal peptide and somatostatin have been shown to inhibit delayed hypersensitivity reactions. A selective release of distinct neuropeptides from different subsets of sensory nerve endings may initiate and modulate contact dermatitis.

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THE ROLE OF NITRIC OXIDE IN SKIN

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Nitric oxide is a novel mediator attracting great interest in many areas of biology. Nitric oxide synthases (NOS) convert arginine and oxygen to citrulline and NO. Besides its vasoactive functions, NO plays a role in immunity and neuronal function. Recent work identified NOS activity in resident skin cells such as keratinocytes, melanocytes, Langerhans cells, fibroblasts and endothelialy cells. Evidence points to a role in inflammatory diseases, particularly psoriasis, antimicrobial functions of skin, response to ultraviolet radiation and autoimmune skin diseases. Pharmacologic intervention in the NO pathway may prove a promising principle in the treatment of inflammatory skin disease.

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VASOAKTIVE INTESTINAL PEPTIDE (VIP) IN EPIDERMAL WOUND HEALING - IN VITRO STUDIES.

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In acute wounds neuropeptides like VIP are release in the wound bed. In neuropeptide deficiencies like diabetes, wound healing is impaired. We wanted to know, wether VIP may be involved in epidermal healing. We investigated the effect of VIP, [D-Phe$_2$]-J-VIP, [Lys-Pro-Arg-Tyr]-VIP, and the VIP fragment [1-12] on induced migration and colonization in vitro. In confluent keratinocyte cultures „wounded" with a razor blade, VIP-treated samples disclosed a more rapid migration from the wound margins. Almost 80 % of the wounded area was covered within 24h. In contrast, VIP-derivates were not significantly different from controls
[$p < 0.02$]. Colonization has been assessed with a polyurethane matrix. In controls, we were able to observe migration of keratinocytes on the matrix within the first 24h. The cells, however, were not able to migrate further to survive. After 48h VIP treated cultures showed a completc colonization of the matrix by keratinocytes vs. less than 10 % of the total area in controls [$p < 0.001$]. These studies show, that VIP may play a pivotal role in epidermal wound healing and that loss of VIP activity may contribute to imapired healing.

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LANGERHANS CELLS AND NEUROIMMUNOCUTANEOUS SYSTEM

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Langerhans cells (LC) are epidermal dendritic antigen-presenting cells. These cells share some properties with nervous cells, such as the expression of S100 protein or neuron specific enolase (NSE) or the production of proopiomelanocortin. Their bone marrow precursors are able to express S100 protein and NSE.

Epidermal LC are closely associated with neurons. Axons are in apposition to LC cell bodies or LC dendrites.When LC lose their contacts with axons, they express PGP9.5, which is a 'specific' neuronal marker.

We have shown that LC express receptors to gastrin-releasing peptide (GRP) or substance P. Granstein's team has demonstrated the presence of receptors to calcitonin-gene related peptide (CGRP) or vasocactive intestinal peptide (VIP).

LC receptors to neuropeptides are functional. Substance P and CGRP inhibit antigen presentation, through the effects of substance P on LC and T lymphocytes and those of CGRP on LC. MSH modulates LC functions probably through cytokines. There is no demonstrated action of GRP. VIP and somatostatin may act on LC too.

When Paul Langerhans described his cells, he thought that the future 'Langerhans' cells had some neural function. This idea was abandoned because it was obvious that LC are immune cells but later it was revealed to be partially true.

The effects of neuropeptides on LC suggest that their agonists or antagonists may be used to treat many skin diseases.

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NEUROPEPTIDES AND SKIN THERAPY

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Neuropeptides (NP) are a heterogenous group of molecules which act in the skin as neurotrasmitters, neuromodulators, neurohormones and hormones. Both NP themselves, or analogues, and NP antagonists can be helpful as therapeutic agents. Few drugs, are topically avalaible other than capsaicin that antagonize the effects of NP. Capsaicin (8- methyl-n-vanillyl-6-nonenamide) excites C fibers and releases tachykinins. Repeated applications on the skin may be helpful in post-herpetic neuralgia, pruritus acquagenicus, neuropeptidergic acral dysesthesia, notalgia paresthetica. The synthetic octapeptide named Peptide T, provided with VIP like activity, has been successfully used in psoriasis. NP agonistic activities are exerted by α-MSH, CGRP analogues and somatostin analogues. α-MSH has a potent antiinflammatory activity in the skin; CGRP has a long-lasting cutaneous vasodilating effect and somatostin analogues can be helpful in treating severe forms of psoriatic arthritis. Finally, the competitive inhibitor of SP, spantide, partially inhibits (73%) the flare caused by histamine and leaves the wheal unchanged. It also suppresses immunologic reactions such as contact urticaria and tubercolin reaction.

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EFFECTS OF NERVE GROWTH FACTOR ON DRG NEUROPEPTIDE SYNTHESIS AND THERMAL NOCICEPTION IN RATS

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Small diameter primary afferent neurons that respond to the neuro-excitant capsaicin (mostly belonging to the group of C-polymodal nociceptors) fulfill a role that exceeds that of impulse transmission to the CNS: Local release of neuropeptides has profound influences on the function of the innervated tissue. In order to adjust neuropeptide supply of afferent nerve endings to increased demand (e.g. in neurons projecting to inflamed tissue), mechanisms must be operative to regulate neuropeptide synthesis in cell bodies of dorsal root ganglia (DRG). There is good evidence that one of these regulatory mechanisms is represented by nerve growth factor (NGF) which is locally synthesized, increased in inflamed tissue, taken up by afferent terminals and transported to the DRG. Our studies have focused on the effects of exogenous NGF on the functional and biochemical properties of rat primary afferent neurons. The results show that systemic administration of NGF (0.1 mg/kg every other day, for 8 d) increases the synthesis of calcitonin gene-related peptide (CGRP) and of tachykinins (TK) in dorsal root ganglia (DRG) as determined 24 h after the last injection. At this time, no change in thermal nociceptive threshold is detectable. However, following each injection of NGF thermal hyperalgesia is present for several hours (<24h). Treatment of rats with compounds that attenuate the injection site hyperalgesia (see below) have no analgesic effect under these conditions. Intraplantar injections of NGF (4 μg) into the rat hindpaw can produce mast cell degranulation, stimulation of leukotriene biosynthesis, and long-lasting leukocyte accumulation. Thermal hyperalgesia that immediately follows local injection of NGF is attenuated by the 5-lipoxygenase inhibitor BAY X1005 and by colchicine, compounds that also inhibit NGF-induced leukotriene biosynthesis. Repeated local injections of NGF over 3 days increase the synthesis of CGRP and of TK in afferent neurons innervating the injection site, but this effect has no detectable consequences on thermal nociception. It can be assumed, therefore, that NGF induces thermal hyperalgesia via several mechanisms, that are not causally related to increased neuropeptide synthesis.

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NERVE GROWTH FACTOR AND THE SKIN

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Human keratinocytes synthesize and secrete nerve growth factor (NGF), which is down-regulated by UVB irradiation. Both keratinocytes and melanocytes express the low- and the high-affinity (trk) NGF- receptor (NGF-R) at the mRNA and at the protein level. Trk in keratinocytes and in melanocytes becomes phosphorylated within minutes after the addition of NGF. NGF stimulates keratinocyte proliferation in a dose-dependent manner and this mitogenic activity is inhibited by the natural alcaloid K252, a specific inhibitor of NGF- induced trk phosphorylation. We have shown recently that NGF induces the up-regulation of NGF mRNA in human keratinocytes. Moreover, autocrine NGF is capable of protecting keratinocyte from spontaneous apoptosis, since the addition of K252 or anti-NGF antibody to the medium, in absence of exogenous NGF, induces keratinocyte apoptosis. K252 and anti-NGF-induced apoptosis is paralleled by the down-regulation of bcl-2. NGF, released from keratinocytes, exerts paracrine effects on melanocytes, in particular it regulates melanocyte dendricity and migration. In addition, NGF prevents UV-induced melanocyte apoptosis by up-regulating bcl-2. Finally, the low-affinity NGF-R (p75) seems to be responsible for melanocyte apoptosis.

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A NEW ROLE FOR NEUROTROPHIN-3 AND BRAIN-DERIVED NEUROTROPHIC FACTOR IN SKIN: INVOLVEMENT IN THE CONTROL OF HAIR FOLLICLE CYCLING.

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Increasing evidence suggests that neurotrophins not only control the development of skin innervation, but are also involved in the regulation of tissue morphogenesis and remodelling. Here we have studied the expression and functions of neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF) during the murine hair cycle. In the back skin of C57BL/6 mice with all follicles in telogen, NT-3 protein levels reached 0.4 ng/mg, while BDNF gene expression was practically absent. Anagen development was accompanied by a decline of both NT-3 mRNA and protein levels. However, maximal levels of NT-3 and BDNF transcriptions, and of NT-3 protein content (1 ng/mg) were found during synchronized hair follicle regression. In early catagen hair matrix keratinocytes (KC) expressed maximal BDNF mRNA, BDNF-, TrkB-, NT-3-, and TrkC-immunoreactivity (IR). Dermal papilla fibroblasts were TrkB+, NT-3+, and TrkC+. During late catagen, all of these antigens disappeared from the dermal papilla, while KCs of the regressing epithelial strand were TrkB+, NT-3+, and TrkC+, and those of the secondary hair germ were BDNF+, NT-3+, and TrkC+. NT-3 and BDNF promoted catagen development in murine skin organ culture. Precocious catagen development was found in neonatal NT-3- or BDNF- overexpressing transgenic mice, while catagen retardation was seen in heterozygous NT-3 (+/-) and homozygous BDNF knockout (-/-) mice, compared to corresponding wild type mice. NT-3 and BDNF may be important control elements in the regulation of catagen, and TrkC and TrkB agonists/antagonists may be exploited therapeutically as novel hair growth-modulatory drugs for the management of hair growth disorders.

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NEUROPEPTIDES AND NGF IN VASOSPASM. Pauline M Dowd, University College London Medical School, London, UK.

In primary Raynaud's phenomenon (1°RP), RP secondary to systemic sclerosis (2°RP) and to occupational exposure to vibration (vibration white finger (VWF)), we have demonstrated by immunohistochemistry a significant reduction in the number of calcitonin gene-related peptide (CGRP)-containing sensory-motor nerve fibres in digital skin and also a reduction in the total number of nerves staining with the pan neuronal marker protein gene product 9.5 (PGP 9.5). In 1°RP and 2°RP the reduction in the PGP 9.5 immunostaining can be accounted for solely by the reduction in the CGRP immunostaining, but in VWF the deficit in PGP 9.5 staining is too great to be accounted for solely by the reduction in CGRP staining indicating a more generalized neuronal loss. Abnormal responses to intradermal injections of endothelin-1 and histamine have provided pharmacological evidence for a functional significance in vivo which is in concordance with the immunohistochemical findings.

Nerve growth factor (NGF) is necessary for the survival and differentiation of sensory and sympathetic neurones during development. In adult rats there is evidence that it has a role in the transmission of itch and pain and in collateral sprouting of sensory-motor nerves following denervation but not in nerve regeneration following crush injury. Both collateral sprouting and nerve regeneration are associated with increased expression of growth associated protein 43 (GAP 43).

In digital skin biopsies from patients with 2°RP and VWF there increased intensity of staining for NGF in the epidermal cells. GAP 43 immunostaining was reduced in VWF and increased in 2°RP

In patients with VWF the decrease in GAP 43 immunostaining may be a reflection of an underlying inability to repair vibration-induced damage despite increased NGF expression whilst the increased GAP 43 immunostaining in 2°RP indicates that the fibres other than CGRP-containing fibres may perhaps be proliferating.

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NEUROPEPTIDES IN SKIN INFLAMMATION

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There is an evidence from many recent studies that neuropeptides such as substance P, vasointestinal peptide (VIP), Calcetonin-gene-related product (CGRP) as well as proopiomelanocortin(POMC)-peptides such as α-melanocyte-stimulating hormone (αMSH) are present in the dermal as well as epidermal compartment of the skin. The production of these peptides is upregulated upon injurious stimuli including microbial agents, tumor promoters or UV-light. Recently, keratinocytes were detected to express receptors specific for POMC-peptides and αMSH was found to modulate keratinocyte proliferation and cytokine production. Neuropeptides also were identified as potent mediators of immunity and inflammation. Accordingly, substance P, CGRP and αMSH were found to inhibit the induction as well as elicitation phase of a contact hypersensitivity reaction and to induce hapten-specific tolerance in mice. These peptides also downregulate the production of proinflammatory and immunomodulating cytokines such as IL-1 and IL-12 and upregulate the release of suppressor-factors such as Interleukin-10. Furthermore, the function of antigen-presenting cells which recently were demonstrated to express specific receptors for αMSH is downregulated by αMSH. These findings suggest that a complex network of mediators including cytokines and neuropeptides plays a crucial role in the pathogenesis of inflammatory and hyperproliferative skin diseases.

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SKIN POMC PEPTIDES AND ACTIVATION OF THE HUMAN MC1 RECEPTOR

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The melanocortin receptor (MC1R) is expressed on melanocytes where it has a key role in regulating pigmentation phenotype and skin tanning. α-Melanocyte stimulating hormone (α-MSH) is normally considered to be the major ligand at the human MC1R although recent studies suggest that ACTH1-39 is also a potent agonist. These peptides are produced from pro-opiomelanocortin (POMC) in the pituitary and in the skin by several cell types including keratinocytes and melanocytes. Their concentrations in the epidermis exceed those in the circulation suggesting that in the skin locally produced POMC peptides have more relevance in regulating pigmentary responses than their pituitary derived counterparts. Using HPLC we have identified desacetyl-, monoacetyl- and diacetyl α-MSH and several ACTH peptides including ACTH1-39, ACTH1-17 and ACTH1-10 in human epidermis. The concentrations of immunoreactive ACTH were higher than those of α-MSH and the most abundant peptide was ACTH1-17. Its ability to interact with the human MC1R was studied in HEK293 cells stably transfected with the receptor. The peptide showed high affinity for the human MC1R with a Ki of 0.21nM which was similar to that of α-MSH. Receptor coupling was studied in the same cells and it was observed that ACTH1-17 activated adenylate cyclase with an EC_{50} of 0.43nM. In the same assay α-MSH gave a value of 1.08nM. The effects of ACTH1-17 was also examined in cultured human melanocytes. As with α-MSH not all cultures responded but in those that did ACTH1-17 increased dendricity and the melanin content of the cells. However, the melanin dose response curves for ACTH1-17 were biphasic with two EC_{50} values of 0.01 and 80pM both of which were lower than the single value of 100pM for α-MSH. These results demonstrate that ACTH1-17 is a more potent agonist at the human MC1R than α-MSH and is capable of stimulating melanocyte dendricity and melanogenesis. α-MSH is therefore not the only POMC peptide to stimulate human melanocytes. Since ACTH peptides also have this effect and are present in the epidermis in greater abundance than α-MSH the possibility should be considered that they too have a role in regulating human melanocytes and hence skin pigmentation.

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Plasma levels of β-endorphin immunoreactivity are increased in tanned subjects.

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Previously we reported (Wintzen *et al.* J Invest Dermatol 106:673-678, 1996) the detection by western blotting, of POMC and its derivatives β-lipotropin (βLPH) and β-endorphin (βE) by human keratinocytes (Kc) *in vitro*. Despite the multiple reports on POMC peptides in skin however, we have not been able to detect βE-immunoreactivity (βE-IR) by radioimmunoassay (RIA) in HPLC-fractionated Kc cell extracts and conditioned media, even though several culture conditions were tested. This finding questions the nature and presence of POMC peptides in skin.

Now we report on the presence of βE-IR (representing βE and/or βLPH) *in vivo*, by RIA. Three male subjects with skin types III were exposed to 15J/cm2 UVA, and βE-IR levels in vycor-extracted plasma were determined before, and up to 1 hour after irradiation. These measurements were carried out once in late spring, and twice at the end of summer. No βE-IR could be detected in skin exposed to solar simulated UV (cumulative dose of 1920mJ) or UVA. Neither were any significant changes observed in βE-IR plasma levels, in the course of 1 hour following UVA irradiation. However, basal levels of βE-IR at the end of summer, when subjects were darkly tanned, were significantly higher than basal levels in spring (60 *vs* 27 pg/ml, respectively), when subjects had no tan at all. These preliminary results are in accordance with an earlier report that plasma levels of αMSH-IR, another POMC product, are twice as high in summer than in winter.

Based upon our in vitro results its seems unlikely that the rise in plasma βE-IR originates from the skin. Rather, the pituitary is the likely source of this βE-IR. It may be of interest to consider that the increased level of βE-like peptide in human plasma after prolonged exposure to UV (sun) light may be related to the feeling of well-being that often accompanies sunbathing.

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INFLUENCE OF NALTREXON ON HISTAMINE AND ACETYLCHOLINE INDUCED ITCHING AND ALLOKNESIS Gisela R. Heyer Dept. of Dermatology, *Inst. of Physiology, University of Erlangen, Erlangen, Germany.

Morphine antagonists are known to reduce itching in several pruritic disorders such as pruritus caused by cholestasis. Therefore, we studied the effect of naltrexon (nemexin®, =NAL), a morphine antagonist, compared to a placebo and an H1-receptor antagonist to determine whether NAL can diminish histamine (=HIS) induced pruritus and alloknesis (= "itchy skin"). Alloknesis, which means that normally non-pruritogenic stimuli cause pruritus, results from central nervous interactions rather than changes in peripheral nervous excitability. Either a placebo or 25 mg NAL were given orally in a double blind manner 60 min prior to the HIS stimulus applied to the forearm in 14 healthy volunteers. In a second, otherwise identical experiment, a placebo or the H1 blocker, 10 mg cetirizin, were orally applied 12 hours before the experiment. Acetylcholine (=ACH), known to cause itching in atopic eczema instead of burning pain in healthy controls, was i.c. injected in 5 atopic eczema subjects, who reported this paradoxic itching after ACH. The area of alloknesis was obtained by stroking the surrounding skin with a brush in centripetal direction to the HIS or ACH application. HIS or ACH induced vasoreactions, intensity of itching (using VAS) and the area of alloknesis were continuously determined for 25 min. NAL and placebo had no effects on the histaminergic or cholinergic vasoreactions. NAL, but not the placebo abolished alloknesis after HIS almost totally and 8 of 14 volunteers developed no alloknesis at all after NAL. NAL and cetirizin, but not the placebo diminished HIS induced pruritus significantly. ACH induced itching was only slightly reduced by NAL, however, alloknesis was suppressed. These findings show, that NAL has a more centrally than peripherally located antipruritic action.

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NEUROPEPTIDES INDUCE NITRIC OXIDE SYNTHASE EXPRESSION AND NITRIC OXIDE PRODUCTION IN HUMAN DERMAL ENDOTHELIAL CELLS

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Recent observations suggest that human dermal endothelial cells (EC) and keratinocytes (KC) are able to synthesyse nitric oxide (NO), one of the key molecules of inflammation, by their specific NO synthases (NOS). Since neuropeptides (NP) are believed to modulate the course of several cutaneous inflammatory reactions, we studied the effects of calcitonin gene-related peptide (CGRP) and substance P (SP) on the inducible (i) NOS mRNA expression and NO production of endothelial cells and keratinocytes.

EC and KC were incubated with different concentrations (10^{-6}-10^{-10} M) of SP and CGRP. After total RNA isolation cDNA was prepared and RT-PCR was carried out with primers specific for the human iNOS. NO production was monitored by measuring the nitrite concentration in the cell-supernatants using a modification of the Griess-method. According to our results, in EC both CGRP and SP induced concentration-dependent iNOS mRNA expression. However, we have not observed increased NO production after per se NP incubation. On the other hand, when ECs were coincubated with IL-1α and CGRP (but not with SP), high-output NO synthesis was detected, which was proportional to the concentration of CGRP. In KCs neither SP nor CGRP caused iNOS mRNA expression or induced NO production.

Our observations provide further evidence that the nervous system might exert direct regulatory effects in cutaneous inflammatory reactions.

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VIP-ergic autocrine loops in local blood flow regulation

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VIP is known as a regulator for the development of the CNS. The neonatal period of brain development is characterised by rapid cellular proliferation in parallel with neuronal differentiation. We examined the expression of native VIP and the VIP receptor associated protein by immunohistochemistry as well as the expression of VIP mRNA by *in situ* hybridisation in the brain stem of newborn piglets. We found both mRNA and VIP as well as the receptor in endothelial cells of veins, arteries and capillaries in the marginal zone of brain stem tissue as well as in pial vessels. The coexpression of native VIP, VIP mRNA and the VIP receptor associated protein within the endothelium suggests the presence of an autocrine loop, which has been detected so far only in neuroblastoma cells. This expression pattern gives evidence to the immaturity of endothelial cells at birth and the presence of an adaptive response in the VIP regulated system during the change from intra- to extrauterine life.

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INDUCIBLE-NITRIC OXIDE SYNTHASE GENE EXPRESSION IN LOCALIZED SCLERODERMA AND KELOID

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Nitric Oxide (NO) is a free radical, produced from L-arginine and oxygen by the enzyme NO synthase, provided with a wide spectrum of activity including vascular smooth muscle tone control inducing vasodilatation in arteries, arterioles and veins, leukocyte and platelet adhesion inhibition, immunemodulation, oxidative tissue damage and cell proliferation. Three genetically distinct nitric oxide synthase (NOS) isozymes have been identified. Isoforms I and III, calcium-dependent, constitutively expressed, rather specific respectively for neurons and endothelial cells and, isoform II or cytokine-induced NO synthase (iNOs), calcium-independent, inducible in macrophages and other cells by bacterial lipopolysaccharide (LPS) and/or some specific cytokines and upregulated by mediators of inflammation. In the present study we investigate iNOs mRNA transcription in 5 biopsies from early phase lesional skin of patients with scleroderma and in 2 skin specimens from non lesional skin. Skin specimens from 2 clinically active keloids have been also investigated performing nonradioactive in situ hybridization procedure using antisense biotin-labelled oligonucleotide probe. In our study we detected iNOs mRNA strongly expressed in perivascular mononuclear cells and epidermal cells in the lesional skin of patients with scleroderma. Keloids showed perivascular cells positively stained. iNOs gene expression was not evidenced in non lesional skin and in control samples.

iNOs mRNA transcription in these fibroproliferative disorders needs to be further evaluated since NO could play a role in maintaining blood supply by non endothelial source but its role in cellularity and matrix deposit in wound healing has been also hypothesized.

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SEROTONIN- AND HISTAMINE-INDUCED PRURITUS AND CUTANEOUS REACTIONS IN 5-HT_3-RECEPTOR BLOCKER PRETREATED SUBJECTS BEFORE AND AFTER MAST CELL DEPLETION

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5-HT_3 receptor antagonists have been reported to be a novel therapeutic principle for the treatment of cholestatic and uremic pruritus. In a previous study we could not verify this effect in healthy volunteers under experimental conditions but we showed that there is a possible interaction of mast-cell-stored histamine and serotonin. In this new study, we investigated the effects of tropisetron, a 5-HT_3 receptor blocker, on histamine- and serotonin-induced itch before and after mast cell depletion with compound 48/80 in 10 healthy volunteers. The results were compared to histamine and serotonin reactions in non-pretreated skin and after pretreatment with an orally applied antihistamine (cetirizine). Wheal and flare areas were planimetrically evaluated. Itch and other sensations were entered on a scale over 24 minutes. The examination also comprised alloknesis, induction of perifocal itch sensation by usually non-itching stimuli. In compound 48/80 pretreated skin, serotonin-induced flares were smaller and itching was significantly weaker compared to serotonin iontophoresis. Wheals were significantly smaller, alloknesis and itch were reduced when mast cell depletion was performed in tropisetron pretreated skin. In compound 48/80 pretreated skin histamine-induced flares were significantly smaller but tropisetron could not effect histamine-induced reactions significantly. When mast cells were depleted, the antihistamine reduced serotonin-induced flares, itch and alloknesis and histamine-induced itch and alloknesis. We conclude that serotonin-induced cutaneous vasoreactions and the effects of 5-HT_3 receptor antagonists are not independent of mast cell functions.

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The Level of Enkephalin in Psoriasis after Treatment with Heliobalneotherapy. Judith B. Nissen, W. Avrack, Estrid S. Hansen, Knud Kragballe, MD, Dept. of Dermatology, Marselisborg Hospital, Aarhus, Denmark, Skin Clinic, Copenhagen, Denmark, Dept. of Pathology, University of Aarhus, Aarhus, Denmark

Enkephalins belong to the opioid peptides known to modulate inflammatory responses and cellular proliferation and differentiation. Earlier, we have shown that enkephalins are present in an increased amount in psoriatic lesions. The purpose of the present study was to determine the effect of heliobalneotherapy on the enkephalin level in psoriatic skin. Ten patients were treated at the Dead Sea for 4 weeks, and keratome biopsies were obtained before and after treatment. The amount of extracted enkephalin in the biopsies was measured by radio-immunoassay. Despite complete clearance of psoriasis after heliobalneotherapy, there was only a slight reduction in the amount of enkephalin (21%). Furthermore, the level of enkephalin was high in uninvolved psoriatic skin after heliobalneotherapy. Immunohistochemical staining of punch biopsies showed that the enkephalins were present in both keratinocytes and monocytes/lymphocytes in involved as well as uninvolved skin. These findings might result from a stimulatory effect of UV irradiation on skin enkephalin levels. Therefore, normal persons were exposed to a single dose of UVA and UVB light (2 MED). UVA, but not UVB irradiation resulted in an increased enkephalin level in normal skin. In conclusion, we found that heliobalneotherapy resulted in only a slight reduction of enkephalin levels in involved skin, despite complete clearance. This may be due to a direct stimulatory effect of UVA irradiation on enkephalin levels in the skin.